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(54) Title: ISOFLAVONES AND RELATED COMPOUNDS, METHODS OF PREPARING AND USING AND ANTI- OXIDANT COMPOSITIONS CONTAINING SAME (57) Abstract Isoflavones and related compounds useful as antioxidants and in antioxidant compositions including edible fats and oils. Many of these compounds can be recovered from tempeh, a fermented soybean product. Others can be prepared by chemical modification of those recovered from tempeh. Additionally, all of the compounds can be chemically synthesized. The compounds of the present invention may be used to provide enhanced stability for a wide range of substances subject to oxidative deterioration including edible food products, oils and fats.		

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ISOFLAVONES AND RELATED COMPOUNDS, METHODS OF PREPARING
AND USING AND ANTIOXIDANT COMPOSITIONS CONTAINING SAME

BACKGROUND OF THE INVENTION

This is a continuation-in-part of application Serial No.

5 804,594, filed June 8, 1977, the disclosure of which is hereby
incorporated by reference into the present disclosure.

Many food products containing and including edible fats and
oils, i.e., fats and oils of animal and vegetable origin or
10 modified fats and oils of animal and vegetable origin, become
rancid or have an undesirable taste and/or color imparted
thereto during storage, especially upon exposure to or on con-
tact with oxygen. A number of chemical compounds have been
employed for avoiding or reducing these effects so that food
15 products containing fats or oils may be kept for longer per-
iods of time, but such agents have not been entirely satisfac-
tory or effective in many cases. Furthermore, such chemical
compounds are usually synthetic chemical products not derived
from or identical with material of natural food classifica-
20 tions and, as a consequence, there has been some question as
to the advisability of using such compounds in food composi-
tions.

Principal antioxidants of the above kinds heretofore employed
25 included BHA (butylated hydroxyanisole), BHT (butylated hyd-
roxytoluene) and TBHQ (tertiary butylhydroquinone), as well
as some other chemicals of which one example is propyl gal-
late (PG). While these materials have been quite effective
in animal fats, such as lard, they are much less useful in
30 some other applications. Their volatility and tendency to
decompose at high temperatures makes them not entirely suit-
able for deep fat fried foods. Indeed, their usefulness for
stability of vegetable oils is less than satisfactory. For
example, they are not entirely effective in protecting against
35 off-flavor development, such as the so-called reversion flav-
or, that occurs, with passage of time, in soybean oil. For
these and other reasons, there has been a need for improvement
in the field of antioxidants, especially those to be used with



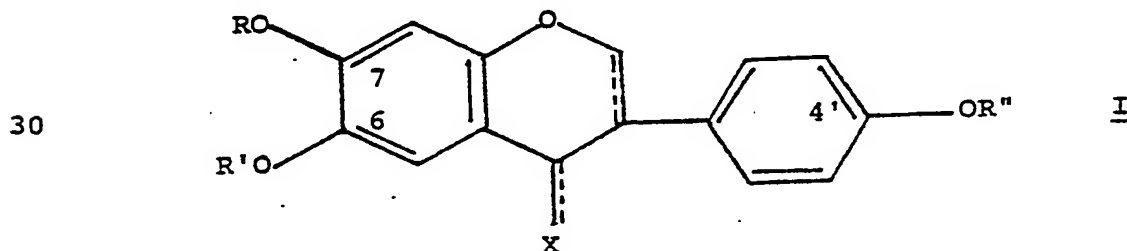
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food materials that comprise or consist of fats or oils.

It has been heretofore known that antioxidant properties are possessed by tempeh, a fermented soybean product obtained by fermenting soybeans with a fungus, either Rhizopus oligosporus or Rhizopus oryzae. Food products containing tempeh, such as fish or fatty meat products exhibit improved stability, see U.S. Patent 3,681,085 (1972). Further, it has heretofore been found that by extracting tempeh with a mixture of hexane and methanol, a component of tempeh, namely oil of tempeh, can be recovered, see U.S. Patents 3,762,933 (1973) and 3,885,256 (1974), which exhibits enhanced antioxidant properties relative to those of tempeh. This oil of tempeh has been found to be useful in stabilizing a wide variety of edible oils and fats.

SUMMARY OF THE INVENTION

The present invention involves the discovery that numerous isoflavones and related compounds which possess antioxidant properties may be recovered from tempeh. Other compounds which also are useful as antioxidants and/or as components of antioxidant compositions can be prepared by chemical modification of those recovered from tempeh. Additionally, all of the compounds can be chemically synthesized. The compounds of this invention have the structure:



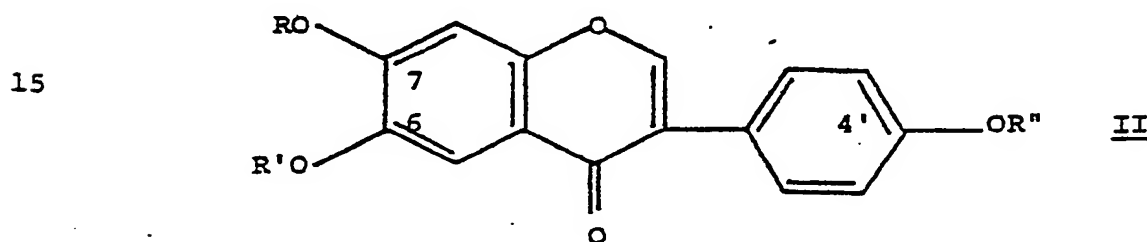
35 wherein the dashed lines may be carbon-carbon single bonds or

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carbon-carbon double bonds, and wherein X may be two hydro-
gen atoms or oxygen, and further wherein each of R, R' and
5 R" may be a methyl or ethyl group or hydrogen.

These compounds possess antioxidant properties and may be uti-
lized in the stabilization of a wide variety of food products
10 including edible fats and oils.

Of these compounds those having the structure



20 may be recovered from tempeh either individually or as compo-
nents of a mixture.

Of the compounds so recovered certain are known compounds,
such as for example, texasin (6,7-dihydroxy-4'-methoxyisofla-
vone), genistein (5,7,4'-trihydroxyisoflavone), daidzein (7,
25 4'-dihydroxyisoflavone), glycitein (6 methoxy-7,4'-dihydroxy-
isoflavone), and the so-called "Murata" compound (6,7,4'-
trihydroxyisoflavone). However, the fact that these compounds
possess antioxidant properties is a new discovery. Of these
30 compounds, texasin has been found to be a particularly effec-
tive antioxidant.

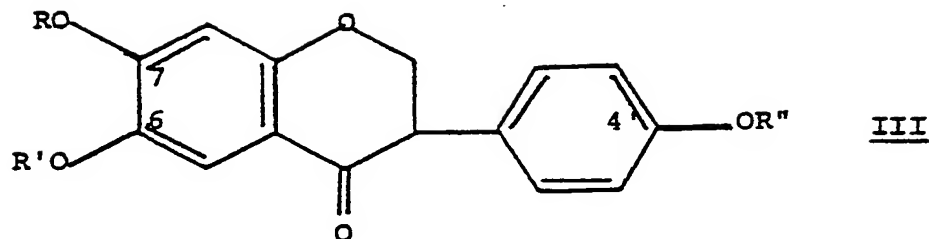
Also, certain compounds having the structure

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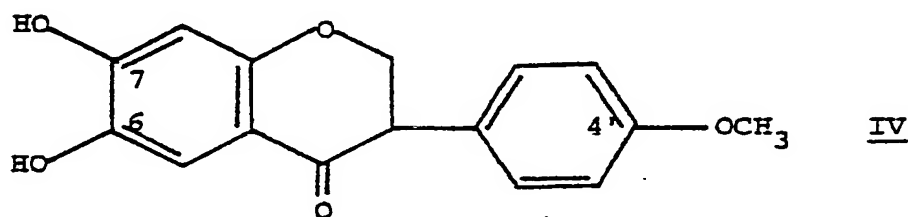
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10 may be recovered from tempeh in minor amounts. However, these compounds are obtained in higher yield upon chemical modification, specifically hydrogenation, of compounds II. Of the compounds III, the compound having the structure

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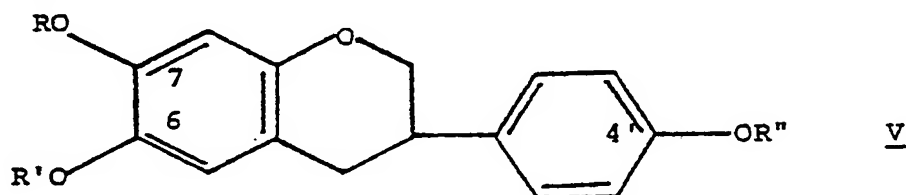


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has been found to be particularly effective. All of the compounds III are novel.

Compounds having the structure

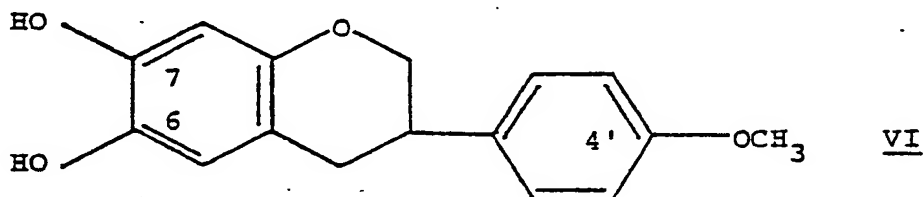
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may be prepared by hydrogenation of Compounds IV. Of these, the compound having the structure

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is the most effective of the antioxidants which have been evaluated. All of the Compounds V are also novel.

5 These Compounds I through VI may be chemically synthesized as well as recovered from tempeh. Thus, in addition to providing compounds useful as antioxidants and in antioxidant compositions, the present invention provides methods of preparing and/or recovering the compounds disclosed.

10

It is therefore a primary object of the present invention to provide compounds useful as antioxidants.

15 It is a related object to provide antioxidant compositions which include these compounds.

It is a further related object to provide edible oils, fats and other food products which include these antioxidant compositions.

20

It is another object of this invention to provide methods of stabilizing edible oils, fats or foods by including in such oils, fats or foods an effective amount of an antioxidant composition.

25

It is a final object to provide methods of recovering and/or preparing compounds useful as antioxidants.

30 How these and other objects of this invention are accomplished will become apparant upon reading the detailed description of the invention including the examples set forth, and the claims which follow. In at least one embodiment of the present invention at least one of the foregoing objects will be achieved.

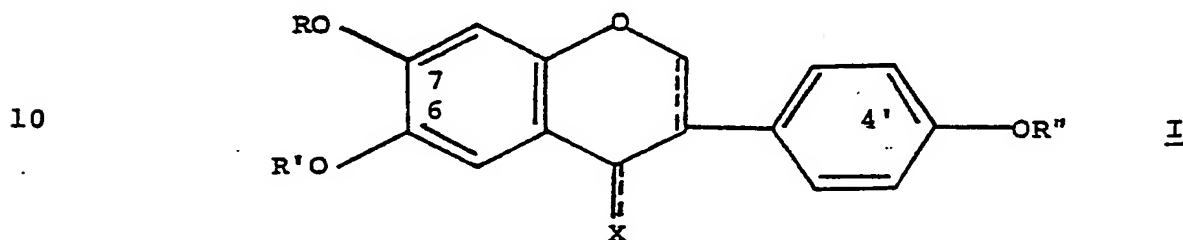
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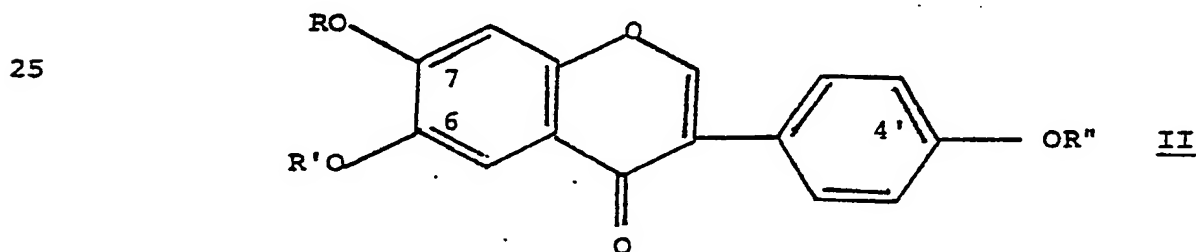
DETAILED DESCRIPTION OF THE INVENTION

In accordance with one embodiment of this invention, isofla-
 5 vones having the structure



15 wherein the dashed lines may be carbon-carbon single bonds
 or carbon-carbon double bonds, and wherein X may be two
 hydrogen atoms or oxygen, and further wherein R, R' and R''
 may be a methyl or ethyl group or hydrogen have been found to
 be useful as antioxidants and as components of antioxidant
 20 compositions.

Certain of these compounds, namely those having the structure



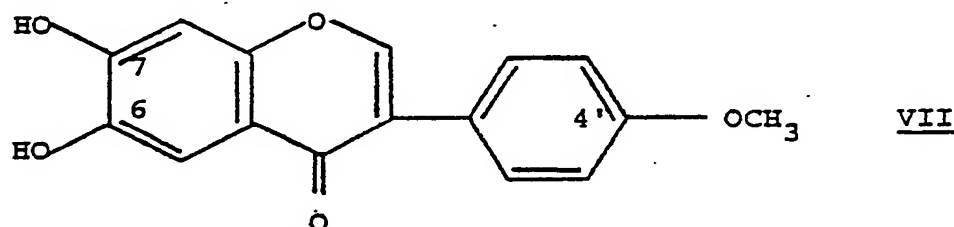
30 are natural products which may be recovered from tempeh, an
 Indonesian foodstuff, by fermentation of soybeans with a fun-
 gus, e.g. either Rhizopus oligosporus ATCC No. 22959 or Rhizo-
pus oryzae ATCC No. 9363. Compounds II are typically present as
 a mixture in tempeh and are most readily recovered therefrom in

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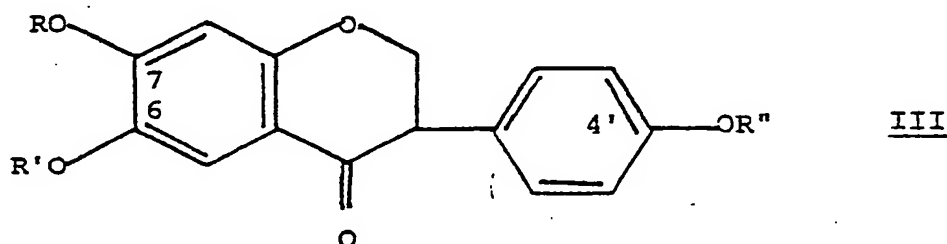
the form of a mixture from which the individual compounds can subsequently be isolated.

5 Compounds encompassed within Structure II as well as within the other structures herein may include stereoisomers and optical isomers. For purposes of this disclosure, no distinction will be made among such isomers so that it is to be understood that the disclosure and claims set forth hereinafter embrace all of the isomers encompassed within the structural formulas indicated. Certain of Compounds II are known compounds, such as for example, texasin (6,7-dihydroxy-4'-methoxyisoflavone), genistein (5,7,4'-trihydroxy isoflavone), daidzein (7,4'-dihydroxyisoflavone), glycitein (6 methoxy-7, 15 4'-dihydroxyisoflavone), and the so-called "Murata" compound (6,7,4'-trihydroxyisoflavone). However, in accordance with the present invention it has been discovered that these compounds possess antioxidant properties. Of these compounds, texasin, which has the structure



has been found to be a particularly effective antioxidant.

30 In addition to Compounds II, additional compounds having the structure

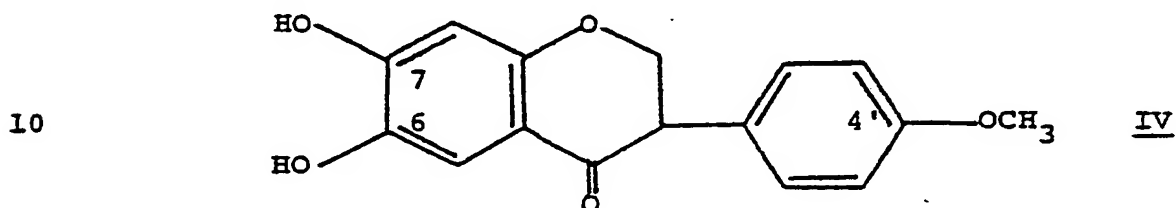


are present in tempeh in minor amounts and may be recovered



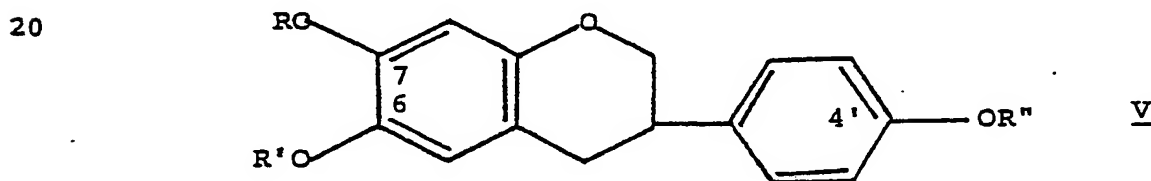
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therefrom. However, these compounds are obtained in higher yield upon chemical modification, specifically, hydrogenation, of Compounds II. Of the Compounds III, the compound produced by hydrogenation of texasin, namely, 6,7-dihydroxy-3-(4-methoxyphenyl)chromanon-4, having the structure



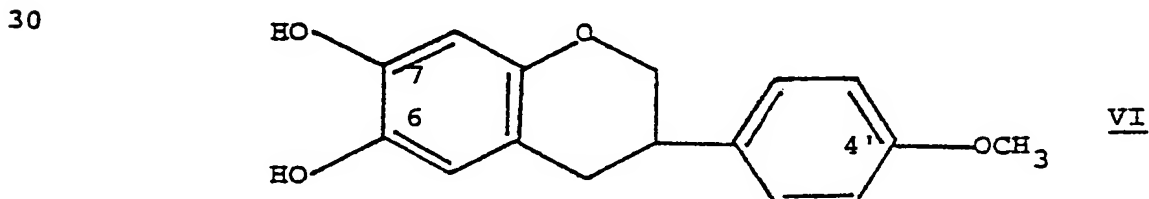
has been found to be particularly effective. Compounds III are novel compounds useful as antioxidants and as components of antioxidant compositions.

Finally, novel compounds having the structure



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may be prepared by hydrogenation of Compounds III. Of these, the compound, 6,7-dihydroxy-3-(4-methoxyphenyl) chroman having the structure



35

is the most effective of the antioxidants which have been evaluated.

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In accordance with another embodiment of this invention, antioxidant compositions may be prepared which include one or more of Compounds I and a suitable carrier. Furthermore, antioxidant compositions which include one or more of Compounds III and/or V provide exceptional antioxidative properties as set forth in the examples hereinafter. Suitable carriers include essentially all non-toxic substances with which the compounds may be admixed or in which they may be dissolved and/or suspended. When the antioxidant composition is intended for use in the stabilization of oils and fats the carrier may be the same or another compatible oil or fat. The amount of the compound or compounds present in the composition may vary within a wide range limited only by the requirement that the amount be effective to provide antioxidant properties to the composition. Typically, amounts will range from about 0.001 to 10 percent by weight.

Stabilized edible fat or oil compositions may be prepared by adding thereto an antioxidant composition which includes one or more of compounds I in an amount effective to stabilize such edible fat or oil. Effective amounts of such antioxidant compositions for improving the stability of oils or fats such as for example, lard, corn oil, olive oil, bean oil, safflower oil, vegetable oil, cottonseed oil, polyunsaturated oils, animal fats or oils and the like are amounts in the range of about 0.01 to 1.0 percent by weight, more or less.

Such antioxidant compositions can also be included in food products to produce stabilized food compositions. Accordingly, food products, such as fish, fatty meat or derivatives thereof, may be stabilized by the addition thereto of an effective amount of an antioxidant composition such as described hereinabove. Effective amounts may typically range from 0.001 to 10 percent by weight, preferably 0.01 to 1.0 percent.

As indicated hereinabove, Compounds II and certain of Compounds III are produced by fermentation of soybeans with a fungus,



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e.g., R. oligosporus or R. oryzae. Compounds II may then be recovered in the following manner. Dry, e.g. lyophilized, tempeh powder or cultured fungus is contacted with a 60-70% aqueous methanol solution for an extended period of time, for example, overnight, at a temperature of about 4°C. thereby producing an extract of methanol-soluble components including one or more of the isoflavones II. The methanol extract solution, after removal of insoluble material, is evaporated to dryness, preferably in vacuo, at an elevated temperature, for example, about 40-60°C. A solid residue is produced most of which is redissolved upon contact with dry methanol. That portion of the residue which is methanol insoluble is separated from the soluble components by centrifugation and discarded. After centrifugation, the methanol supernatant is extracted with hexane several times, for example, two to three times, in order to remove any traces of hexane-soluble impurities, such as lipids. After discarding the resulting hexane extract, the remaining methanol supernatant is evaporated to reduce its volume to a minimal fraction, for example, about 20 ml, and kept at a temperature of about -20°C. for about 15-20 minutes. This results in formation of additional precipitate which is removed and discarded.

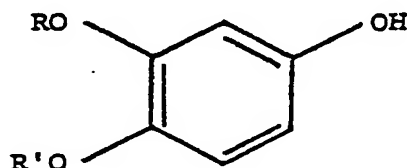
The isoflavones may then be recovered from the methanol supernatant or extract as follows. The supernatant is subjected to molecular sieve chromatography, for example, chromatography on Sephadex LH20 using a suitable size column, for example 2x40cm, and a suitable mobile phase, for example, n-propanol: ethylacetate:water in a ratio 5:5:1. One of the fractions resulting from this chromatographic separation is fluorescent with emission in the blue range of the visible spectrum. This blue fluorescent fraction is separated and subjected to adsorption chromatography on a suitable matrix, for example, silica gel, using an appropriate mobile phase, e.g. ethylacetate:propanol:water = 95:2:3. The resulting blue fluorescent fraction is then rechromatographed on an adsorptive matrix, e.g. thin layer chromatography on silica gel, employing a

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different mobile phase, e.g. cyclohexane:dichloromethane:ethylformate:formic acid = 35:30:30:5. Each of the isoflavones can then be recovered in essentially pure form using its differential mobility on the silica gel plate.

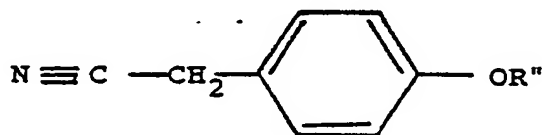
Alternatively, Compounds II can be chemically synthesized by forming a suspension of a compound having the structure

10



15 wherein each of R and R' may be either hydrogen or an ethyl or methyl group and a compound having the structure

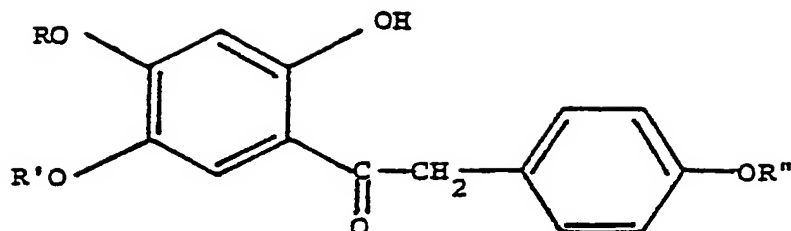
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wherein R'' may be hydrogen, methyl or ethyl in dry ethylether containing zinc chloride. This suspension is then exposed to dry hydrogen chloride for a sufficient time to form an oily product. The suspension is next maintained at an appropriate temperature for a sufficient time to permit the oil product formed upon exposure to dry hydrogen chloride to separate from the suspension. The supernatant remaining after separation of the oil product from the suspension is removed and discarded. Next, a major amount of water and a minor amount of concentrated hydrochloric acid is added to the oil product to form a mixture, the mixture is boiled under reflux for a sufficient time to produce a precipitate product having the structure

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5



10 After recovering this precipitate, it is dissolved in a suitable solvent, borontrifluoride-methyletherate added, the temperature adjusted to about 50°C, methanesulphonyl chloride added as a solution, and then the resulting solution heated at about 90-100°C. for a sufficient time to permit the reaction to proceed substantially to completion. Finally, Compound II is recovered.

Compounds III can be prepared by hydrogenating Compounds II using conventional techniques such as contact with hydrogen gas in the presence of a catalyst under appropriate conditions of temperature, pressure and the like. Similarly, Compounds V can be prepared by hydrogenating Compounds III and/or II.

The following examples are set forth to illustrate the practices of the present invention but are not intended in any way to limit or otherwise restrict the scope thereof as set forth in the preceding description or in the claims which follow.

EXAMPLE I

30 The compounds 6,7-dihydroxy-3-(4-methoxyphenyl) chromanon-(4) and 6,7-dihydroxy-3(4-methoxyphenyl)chroman were evaluated as antioxidants using an automated version of the Swift stability test at 100°C. For a more detailed description of the Swift stability test see A.O.C.S. Tentative Method Cd 12-57 (revised 1959. For the test the lard was heated to 100°C. and air bubbled through at 2 ml/minutes. Every few hours the oil was analyzed for peroxide value using the peroxide value test, A.O.C.S. Official Method Cd8-53 (1960). The tests were car-

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ried out using two different batches of lard. The results of the tests are shown in Table I.

5

TABLE I
AUTOMATED SWIFT STABILITY ON LARD
End of Induction Period in Hours

<u>BATCH</u>	<u>ANTIOXIDANT</u>	<u>CONCENTRATION OF ANTIOXIDANT</u>			
		<u>25 mg/kg</u>	<u>50 mg/kg</u>	<u>100 mg/kg</u>	<u>200 mg/kg</u>
A	6,7-dihydroxy- -3-(4-methoxy- phenyl) chroman			50	62
	6,7-dihydroxy- 3-(4-methoxy- phenyl) chromanon-4			33	38
	BHA			26	32.5
	BHT			27	29
	TBHQ		32	42	
	α -tocopherol (Vitamin E)			21	21
	Blank		+2.5+		
B	6,7-dihydroxy- 3-(4-methoxy- phenyl) chroman	26	43	92	110
	6,7-dihydroxy- -3-(4-methoxy- phenyl) chroma- non-4	15	22	45	62
	BHA	10	30	49	57
	Blank		+6.5+		

35 According to the results obtained, 6,7-dihydroxy-3-(4-methoxy-phenyl)chroman is the most effective of the antioxidants tested. 6,7-dihydroxy-3-(4-methoxyphenyl)chromanon-4 is better than α -tocopherol, but less effective than TBHQ and comparable with



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BHA and BHT.

EXAMPLE 2

5

The Swift stability test described for lard in Example 1 was repeated with palm oil and bean oil. The results again indicated that 6,7-dihydroxy-3-(4-methoxyphenyl)chroman is the most effective of the antioxidants. 6,7-dihydroxy-3-(4-methoxyphenyl)chroman-4 was better than α -tocopherol, but less effective than TBHQ and comparable with BHA and BHT.

EXAMPLE 3

15 The compounds tested in Example 1, namely, 6,7-dihydroxy-3-(4-methoxyphenyl)chroman and 6,7-dihydroxy-3-(4-methoxyphenyl)chroman-4, were also tested to evaluate their effect upon the taste of normal quality lard, bean oil and palm oil. The compounds were added to the lard or oil at amounts ranging
20 from 25-200 mg/kg and stored at 15°C. or 50°C in the dark. The lard or oil was then tasted periodically over a 14-week period. It was found that 6,7-dihydroxy-3-(4-methoxyphenyl)chroman-4 favorably influenced the taste of the lard and oils. Addition of 6,7-dihydroxy-3-(4-methoxyphenyl)chroman
25 imparted an off-taste although this may be due to the presence of an impurity.

EXAMPLE 4

30 The same compounds were also evaluated to determine whether they would induce sterility and/or premature abortion of fetuses by feeding the compounds to female rats. In accordance with the Allen-Doisy Test ovariectomized rats were fed the compounds. No negative effect upon the production of female hormones or antifertility activity was noted. Additionally,
35 feeding of the compounds to pregnant rats produced no abortive action.

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EXAMPLE 5

The compounds 6,7-dihydroxy-3-(4-methoxyphenyl)-chromanon-(4) and 6,7-dihydroxy-3-(4-methoxyphenyl)-chroman were tested in lard using the Swift Stability Test at a series of concentrations. The results for two series of samples are shown in Tables II and III.

10

TABLE IIANTIOXIDANT PROPERTIES OF6,7-dihydroxy-3-(4-methoxyphenyl)-chroman

	<u>Concentration in Stripped lard [2g]</u>	<u>Protection [percent]</u>	<u>POV [meqO₂/kg]</u>	
15	0.5 mg = 250 ppm	96	1.9	
	0.2 mg = 100 ppm	96 98	2.2	1.0
	0.1 mg = 50 ppm	86		6.4
	0.05 mg = 25 ppm	38 33	32.8	30.5
	0.02 mg = 10 ppm	22	41	
20	None	0 0	52.6	45.4

TABLE IIIANTIOXIDANT PROPERTIES OF6,7-dihydroxy-3-(4-methoxyphenyl)-chromanon-4

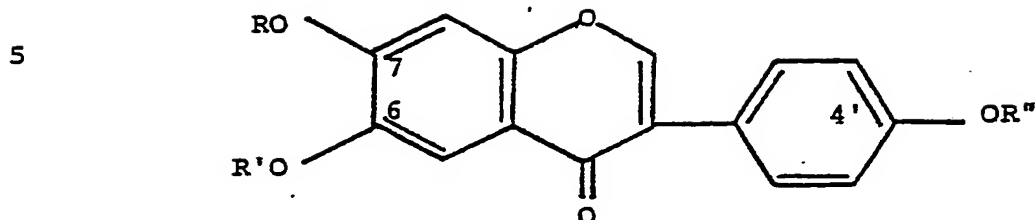
	<u>Concentration in Stripped lard [2g]</u>	<u>Protection [percent]</u>	<u>POV [meqO₂/kg]</u>	
25	0.5 mg = 250 ppm	99	0.5	
	0.2 mg = 100 ppm	94 98	3.1	0.8
	0.1 mg = 50 ppm	53		21.3
30	0.05 mg = 25 ppm	44 25	29.3	34.1
	0.02 mg = 10 ppm	22	41	
	None	0 0	52.6	45.4

EXAMPLE 7

An antioxidant composition was obtained from tempeh which included Texasin and additional isoflavones having the structure



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10 wherein R, R' and R'' may be a methyl or ethyl group or hydrogen. This mixture of isoflavones was evaluated using the Swift Stability Test described hereinabove at 110°C. Lard samples containing 500, 50 and 1 ppm of the mixture were prepared by suitable dilutions of a stock mixture of the isoflavone mixture
 15 dissolved in 1:1 chloroform-methanol and the mixture was evaporated under vacuum to a clear solution. A similar set of lard samples containing BHT was also prepared for comparison. Results of the test are shown in Table IV.

20

TABLE IV

<u>Sample</u>	<u>Concentration Antioxidant (ppm)</u>	<u>Stability (hours)</u>
25 Isoflavone mixture	500	36
	50	22
	1	22
BHT	500	81
	50	35
	1	24
30	1	24
Control	0	22

It may be seen that the isoflavone mixture at 50 ppm and 1 ppm produced no significant increase in stability. With the 500
 35 ppm sample, however, the stability was comparable to that of the 50 ppm BHT sample. Thus, the isoflavone mixture has antioxidant properties, but its activity is less than that of BHT under conditions of the test. It should be noted that

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this test is not completely suitable for evaluating isoflavones under all conditions because of the severity of the conditions used (temperature 110°C. with air bubbled through the sample).

5

EXAMPLE 8

An antioxidant composition was obtained from tempeh which included Texasin and trihydroxy-isoflavone. This mixture of isoflavones was evaluated using the Oven Storage Test described hereinabove at a temperature of 60°C. for 3 days. Peroxide values were determined for stripped lard samples containing the mixture. The results are set forth in Table V.

15

TABLE V

<u>Stripped Lard [g]</u>	<u>Concentration Antioxidant [mg] =</u>	<u>ppm</u>	<u>Protection [percent]</u>
1.0	0.5	250	98
1.5	0.25	125	96
1.8	0.10	50	49

20

25 EXAMPLE 9

A series of isoflavones either recovered from tempeh or chemically synthesized were evaluated using the Oven Storage Test for 40 hours and for 3 days at 60°C. In order to dissolve the isoflavones in 2 kg of stripped lard an emulsifier was employed such as a glycerol. The results are shown in Table VI.

30



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TABLE VI
ANTIOXIDANT PROPERTIES OF ISOFLAVONES

Amount Isoflavone [mg]	Conc. [% lard]	% Protection (40h, 60°C.)							% Protection (3days, 60°C)						
		SAMPLE							SAMPLE						
		1	2	3	4	5	6	7	1	2	3	4	5	6	7
1.0	0.05	21	21	0	100	91	45	67	28	21	31	99	77	50	20
0.5	0.025	-	-	-	95	90	-	57	-	-	-	91	75	-	9
0.1	0.005	0	0	8	43	0	15	17	0	22	46	29	1	2	0
0.05	0.00025	-	-	-	-	-	-	17	-	-	-	-	-	-	0

- 1) 6-methoxy-7,4'-dihydroxyisoflavone (glycitein)
- 2) 7-methoxy-6,4'-dihydroxyisoflavone (kakkatin)
- 3) 6,7-dimethoxy-4'-hydroxyisoflavone
- 4) isoflavone mixture chemically synthesized without #7
- 5) 6,4'-dimethoxy-7-hydroxyisoflavone
- 6) isoflavone mixture from tempeh
- 7) 6,7,4'-trihydroxyisoflavone

20 The test results indicate that each of the isoflavones has anti-oxidant properties. Particularly effective is the isoflavone mixture obtained by chemical synthesis which includes various methoxy-substituted isoflavones. Also, the compound 6,4'-dimethoxy-7-hydroxyisoflavone was particularly effective.

25

EXAMPLE 10

Synthesis of 1,2,4-trihydroxybenzene

Fifty grams (0.46 mol) of 1,4-benzoquinone was slowly added
 30 with continuous stirring and cooling to a mixture consisting of 150 g acetic acid anhydride and 3 ml concentrated sulfuric acid. During this reaction the temperature should be maintained between 40 and 50°C. After all of the quinone has been dissolved and the heat development has ceased, the reaction
 35 mixture was poured into two liters of water and the precipitate formed was filtered off. After drying in a vacuum desiccator one obtained 1,2,4-triacetoxybenzene in the form of a light brown amorphous powder which may be recrystallized from methanol.

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Eighty grams of the crude 1,2,4-triacetoxybenzene were dissolved in 200 ml of methanol containing 6g of concentrated sulfuric acid. The reaction mixture was boiled under reflux for one hour. Then the solution was cooled to room temperature and neutralized with an equivalent amount of fine powdered sodium carbonate. Thereafter 800 ml of ethylether was added whereupon sodium sulfate precipitated out, which was removed by filtration. Reaction by-products and contaminants being responsible for a dark red coloration may be removed employing solid silica gel. After evaporation of the supernatant under reduced pressure, a dark red oil is obtained which crystallized upon standing at 40°C. in the form of a slight reddish solid mass which is pure enough for the next step. This product is 1,2,4-trihydroxybenzene.

EXAMPLE 11Synthesis of 6,7-dihydroxy-4'-methoxyisoflavone (6,7-dihydroxy-3-(4-methoxyphenyl)chromone)

35.4 grams of 1,2,4-trihydroxybenzene were suspended in 200 ml of dry ethylether containing 30g of dry zinc-chloride (0.22 mol) and 50g (0.34 mol) of p-methoxyphenylacetonitrile. The suspension was then exposed for 4 hours at 0°C. to a gentle stream of dry hydrogen chloride (HCl), the gas bubbling through the suspension under continuous stirring. Then the reaction mixture was kept for 70 hours at 4°C. and thereafter the supernatant was decanted from the heavy oil which had separated. The oil was washed twice with ethylether, then one liter of water and a few ml of concentrated hydrochloric acid were added and the mixture boiled for 1 hour under reflux. After cooling to room temperature the precipitate was collected by filtration and recrystallized from ethanol/water. This precipitate was (4-methoxybenzyl)-2,4,5-trihydroxyphenyl ketone.

Four grams (14.6 milimol) of (4-methoxybenzyl)-2,4,5-trihydroxyphenyl ketone were dissolved in 50 ml. of dry dimethylformamide. To this solution was added 7.5 grams of borontri-



-20-

fluoride-methyetherate ($\text{BF}_3-(\text{CH}_3)_2\text{O}$) dropwise. Under spontaneous elevation of the temperature the color of the solution turns to yellowish-green. Then the temperature of the reaction is adjusted to 50°C . and a solution of 5 grams methane-sulphonyl chloride ($\text{CH}_3\text{SO}_2\text{Cl}$) in 25 ml of dry dimethylformamide (DMF) is added dropwise. Thereafter, the solution is heated for 90 minutes at $90-100^\circ\text{C}$. After cooling to room temperature, the reaction mixture is poured into 500 ml water and the resulting yellow precipitate is filtered off. After drying in a desicator the crude product is purified by boiling in 50 ml methanol and then 50 ml of ethyl ether. The resulting white powder can be recrystallized from dioxane or glacial acetic acid. This product is 6,7-dihydroxy-3-(4-methoxyphenyl)-chromone (texasin).

EXAMPLE 12Synthesis of 6,7-dihydroxy-3-(4-methoxyphenyl)chromanon-4

Six grams of texasin (6,7-dihydroxy-3-(4-methoxyphenyl)chromone) were dissolved and partially suspended in 500 ml of ethanol and hydrogenated at normal pressure and room temperature using 10% palladium/charcoal as a catalyst under addition of 6 drops of triethylamine. The catalytic hydrogenation is continued until no starting material is detectable by means of thin layer chromatography. Thereafter, the catalyst is removed by filtration, an equal amount of water is added to the reaction mixture and the solution is evaporated under reduced pressure. After removal of the largest part of the ethanol the product precipitates out in pure form. After filtration and drying in a desicator 5.5 grams of a light yellow powder is obtained which may be recrystallized once from ethanol/water. The product has the formula $\text{C}_{16}\text{H}_{14}\text{O}_5$, molecular weight 286, and melting point 215°C . It was characterized by UV, NMR, IR and Mass spectroscopy and determined to be 6,7-dihydroxy-3-(4-methoxyphenyl)-chromanon-4. The product is actually a mixture of optical isomers but since the isomers exhibit the same properties further characterization was not deemed necessary.



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for the purposes of this disclosure. Therefore it is to be understood that the scope of the disclosure and claims embraces the optical isomers of the compounds discussed herein.

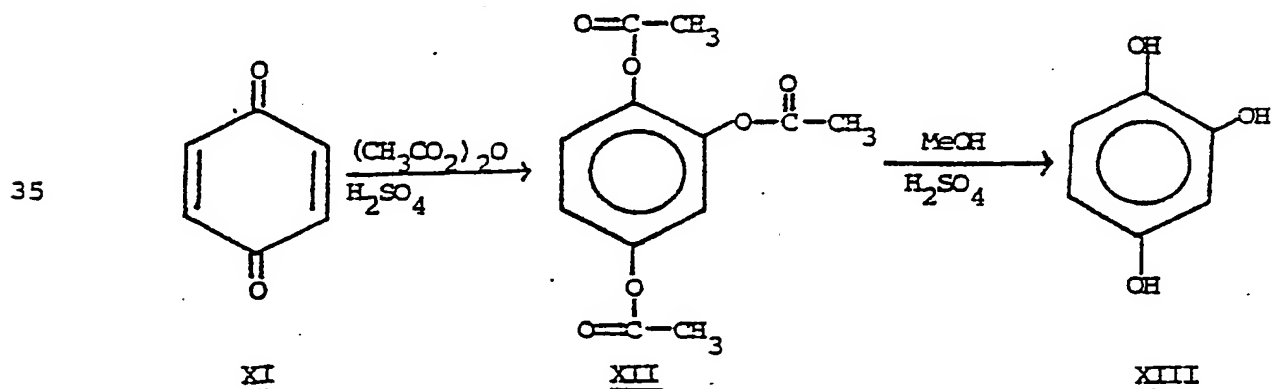
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EXAMPLE 13Synthesis of 6,7-dihydroxy-3-(4-methoxyphenyl)chroman

Fifteen grams of texasin (6,7-dihydroxy-3-(methoxyphenyl)chromone) were dissolved and partially suspended in 500 ml of ethanol and hydrogenated at normal pressure and room temperature using 10% palladium/charcoal as a catalyst under addition of 20 drops of concentrated sulfuric acid. The catalytic hydrogenation is continued until neither texasin nor any of the 6,7-dihydroxy-3-(4-methoxyphenyl)chromanon-4 was detected by thin layer chromatography. The further purification was identical to that described in Example 12. The product has the formula $C_{16}H_{16}O_4$, molecular weight 272, and melting point $160^{\circ}C$. It was characterized by UV, NMR, IR and Mass spectroscopy and determined to be 6,7-dihydroxy-3-(4-methoxyphenyl)-chroman. As in Example 12, the product is actually a mixture of optical isomers but since the isomers exhibit the same properties further characterization was not deemed necessary for the purposes of this disclosure. Therefore, it is to be understood that the scope of the disclosure and claims embraces the optical isomers of the compounds discussed herein.

EXAMPLE 14Synthesis of 6,7-dihydroxy-isoflavone

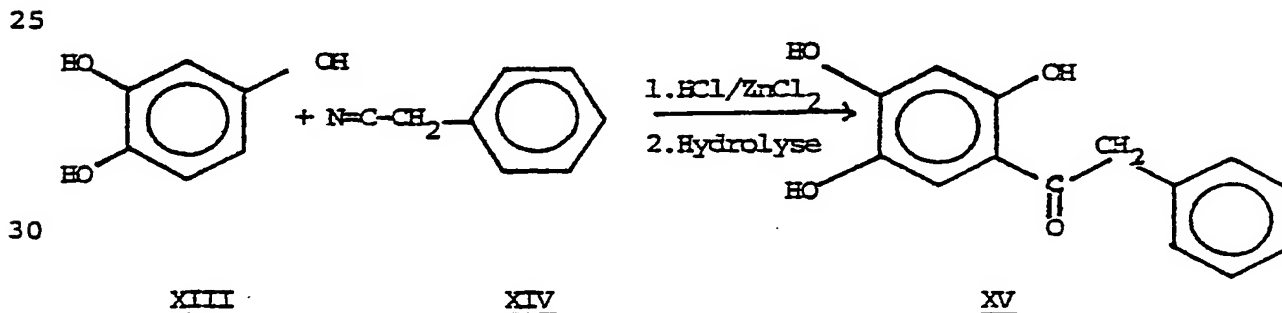
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-22-

50 g (0.46 mol) 1,4 benzoquinone (XI) was slowly added with stirring to a mixture of 150 g of acetic anhydride and 3 ml of conc. sulfuric acid during which the reaction mixture was cooled so as to keep the temperature between 40 and 50°C. After all of the quinone was dissolved and the heat of formation evolved, the reaction product was washed with an excess of water and the precipitate removed by filtration. Upon drying the precipitate 1,2,4-triacetoxybenzene (XII) was obtained as a light brown powder which can be identified as such after crystallization from methanol. The yield from the reaction was 106 g (91%).

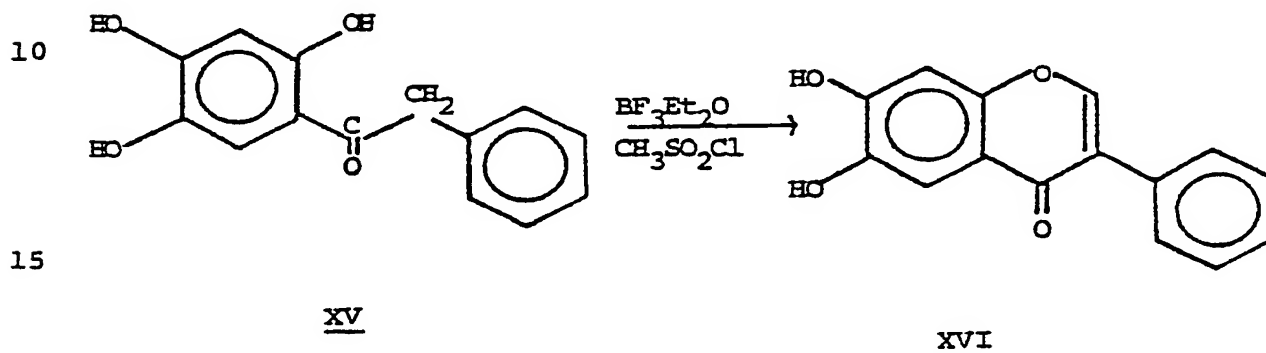
80 g (0.32 mol) of the crude product XII in 200 ml of methanol was heated under reflux for one hour in the presence of 6 g conc. sulfuric acid. The reaction mixture was neutralized with sodium carbonate and then ether added. Na_2SO_4 precipitated out and was separated by filtering. Known contaminants were removed by treatment with silica gel resulting in a solution having a dark red color. Upon distillation this solution yielded a dark red oil which rapidly crystallized to a light reddish solid material (XV). The yield of this reaction was 35.4 g (88%).



35.4 g (0.28 mol) of Compound XIII was suspended with 30 g water-free ZnCl_2 (0.22 mol) in 200 ml dry ether and then mixed with 39.8 g (0.34 mol) benzylcyanide (XIV). Next, dry HCl gas was introduced in the reaction mixture with stirring for four hours at 0°C. After about 70 hours storage in a refrigerator the re-

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maining solution was separated from the oil which had precipitated, washed two times with ether and heated one hour in 1 liter of water with which a few ml of HCl was mixed. After cooling the precipitate was removed by filtering and crystallized from alcohol/water. The yield of Compound XV was 25 g (36.5%).



3.4 g (0.0146 mol) Compound XV was dissolved in 50 ml dry DMF and 7.5 ml borontrifluoride-methyl-etherate added dropwise. Upon heating the solution assumed a yellow-green color. The solution was heated to 50°C and then 5 g of methanesulfonyl chloride in 25 ml of DMF was added. Heating was continued for 90 minutes at 90-100°C and then the reaction mixture was cooled by adding about 0.5 liter water. The resulting yellow precipitate was removed by filtration and after drying yielded 3.1 g of a light brown powder which was purified by crystallization from methanol/water. This provided 3.0 g (81%) of Compound XVI, 6,7-dihydroxy-isoflavone.

EXAMPLE 15

6,7-dihydroxy-isoflavone may be hydrogenated using the methods described in Examples 12 and 13 to prepare the chromanon-4 and chroman respectively. Both of these compounds have been found effective as antioxidants at concentrations as low as 50 parts per million in the standard lard test described in Example 1.



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As will be obvious to one skilled in the art, many modifications, variations, substitutions and other alterations can
5 be made in the practices of this invention without departing from the spirit and scope thereof as set forth in the preceding description and examples or in the claims which follow.



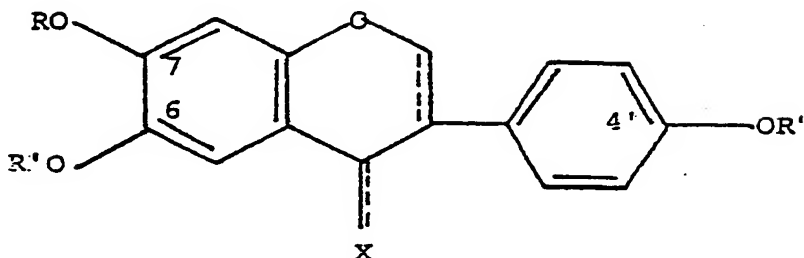
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What is claimed is:

1. A compound having the structure:

5

10

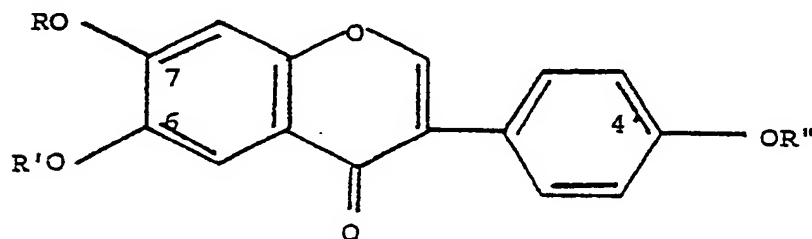


wherein the dashed lines may be carbon-carbon single bonds or carbon-carbon double bonds, and wherein X may be two hydrogen atoms or oxygen, and further wherein each of R, R' and R'' may be a methyl or ethyl group or hydrogen.

20

2. A compound in accordance with Claim 1 having the structure:

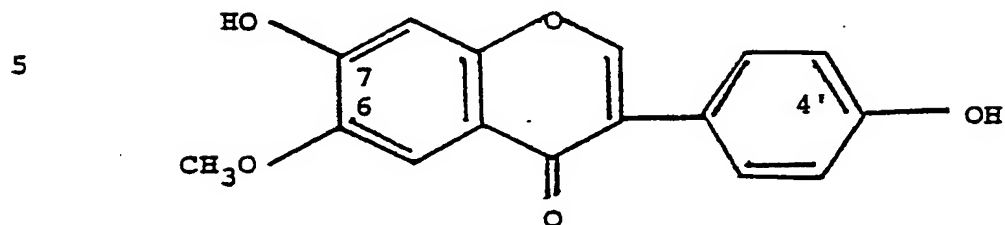
30



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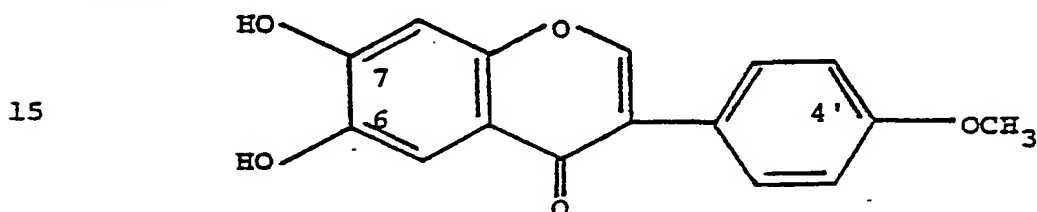
-26-

3. A compound in accordance with Claim 2 having the structure:

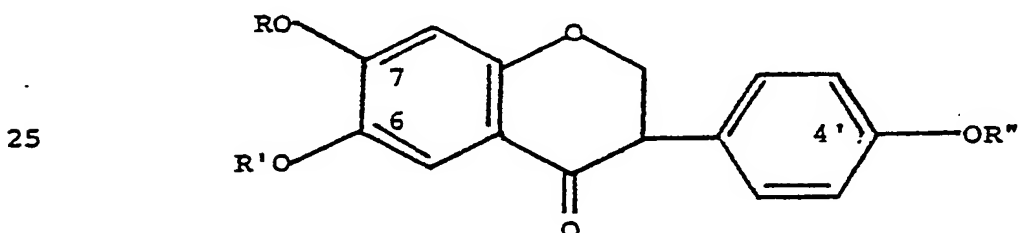


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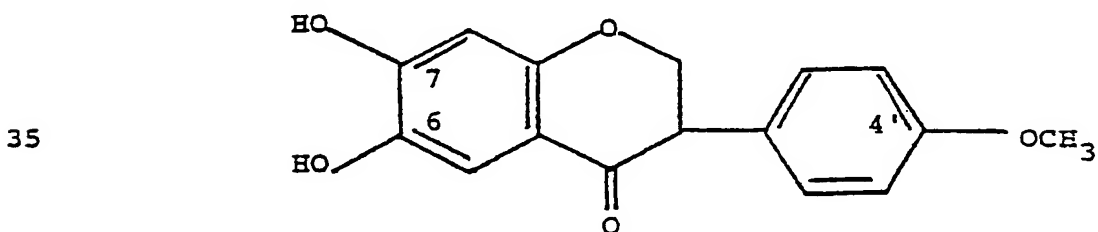
4. A compound in accordance with Claim 2 having the structure:



20 5. A compound in accordance with Claim 1 having the structure:

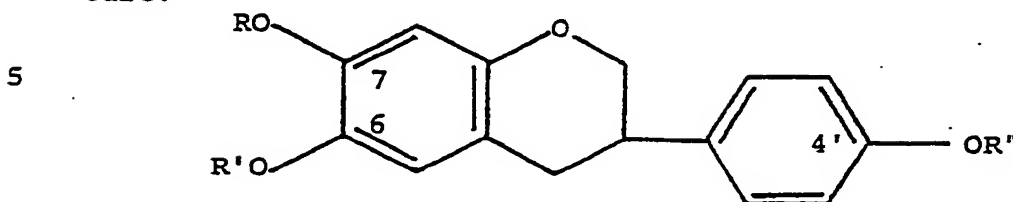


30 6. A compound in accordance with Claim 5 having the structure:

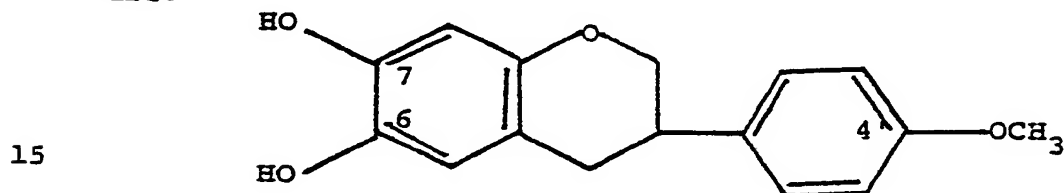


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7. A compound in accordance with Claim 1 having the structure:



10 8. A compound in accordance with Claim 7 having the structure:



20 9. An antioxidant composition which comprises a compound in accordance with Claim 1 and a suitable carrier.

10. An antioxidant composition which comprises a compound in accordance with Claim 2 and a suitable carrier.

25 11. An antioxidant composition which comprises a compound in accordance with Claim 3 and a suitable carrier.

12. An antioxidant composition which comprises a compound in accordance with Claim 4 and a suitable carrier.

30 13. An antioxidant composition which comprises a compound in accordance with Claim 5 and a suitable carrier.

14. An antioxidant composition which comprises a compound
35 in accordance with Claim 6 and a suitable carrier.

15. An antioxidant composition which comprises a compound in accordance with Claim 7 and a suitable carrier.

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16. An antioxidant composition which comprises a compound in accordance with Claim 8 and a suitable carrier.

5 17. A stabilized edible fat or oil composition which comprises an edible fat or oil and an antioxidant composition in accordance with Claim 9, said antioxidant composition being present in an amount effective to stabilize said edible fat or oil.

10

18. A stabilized edible fat or oil composition which comprises an edible fat or oil and an antioxidant composition in accordance with Claim 10, 11, 12, 13, 14, 15, or 16, said antioxidant composition being present in an amount effective
15 to stabilize said edible fat or oil.

19. A stabilized edible fat or oil composition in accordance with Claim 17 wherein said edible fat or oil comprises animal fat or oil, vegetable oil, corn oil, cottonseed oil, lard,
20 olive oil, soybean oil, bean oil, safflower oil, and polyunsaturated oils.

20. A stabilized edible fat or oil composition in accordance with Claim 17 wherein said antioxidant composition is present in an amount from about 0.01 percent to 1.0 percent by
25 weight based upon the weight of said edible fat or oil.

21. A stabilized edible food product such as a fish or meat food product which comprises an edible food product and an antioxidant composition in accordance with Claim 9, said
30 antioxidant composition being present in an amount effective to stabilize said edible food product.

22. A stabilized edible food product such as a fish or meat food product which comprises an edible food product and an
35 antioxidant composition in accordance with Claim 10, 11, 12, 13, 14, 15 or 16, said antioxidant composition being present in an amount effective to stabilize said edible food product.



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23. A stabilized edible food product in accordance with Claim 17 wherein said antioxidant composition is present in an amount from about 0.01 percent to 1.0 percent by weight based upon the weight of said edible fat or oil.

24. A method of stabilizing edible oils, fats or food products which comprises adding to said edible oils, fats or food products an effective amount of an antioxidant composition in accordance with Claim 9.

25. A method for preparing a compound in accordance with Claim 2 which comprises fermenting soybeans, contacting the resulting fermented soybean product with methanol to separate from said fermented soybean product an extract containing said compound and recovering said compound from said methanol extract.

26. A method in accordance with Claim 25 wherein said fermented soybean product is tempeh.

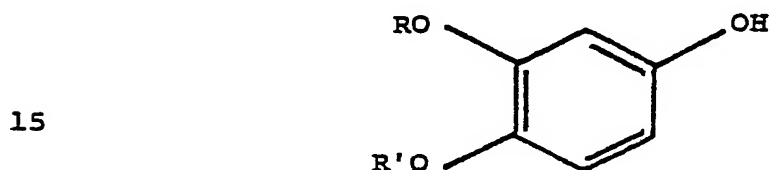
27. A method in accordance with Claim 25 wherein said extract containing said compound is obtained by contacting said fermented soybean product with an aqueous methanol solution at a temperature in the range about 0-10°C., evaporating the resulting methanol extract solution to dryness to produce a residue, redissolving a portion of said residue in dry methanol, separating and discarding the methanol insoluble portion of said residue, contacting said dry methanol solution containing said redissolved residue with hexane to remove contaminating material, discarding the resulting hexane extract, reducing the volume of the remaining methanol extract at a reduced temperature for 10-30 minutes so as to produce a precipitate, removing and discarding said precipitate, and recovering said compound from the remaining extract.



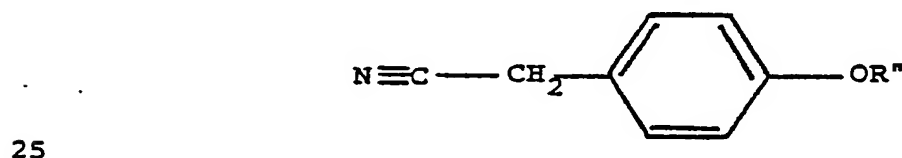
-30-

28. A method in accordance with Claim 25 wherein said recovery of said compound from said methanol extract comprises chromatographic separation, said chromatographic separation
5 being molecular sieve chromatography followed by adsorption chromatography.

29. A method of preparing a compound in accordance with Claim 2 which comprises forming a suspension of a compound
10 having the structure:

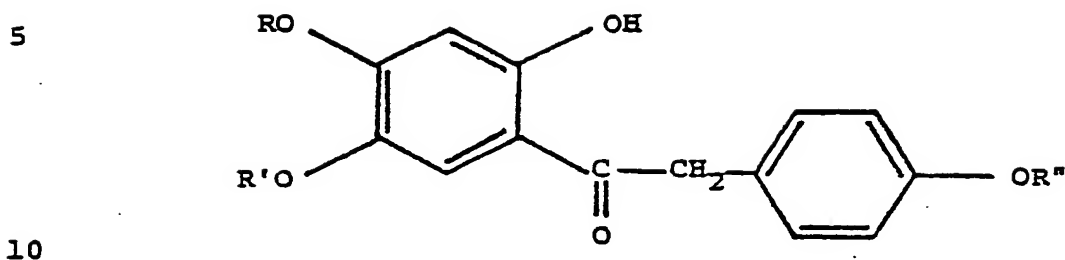


wherein each of R, R' and R'' may be either hydrogen or an ethyl or methyl group and a compound having the structure:
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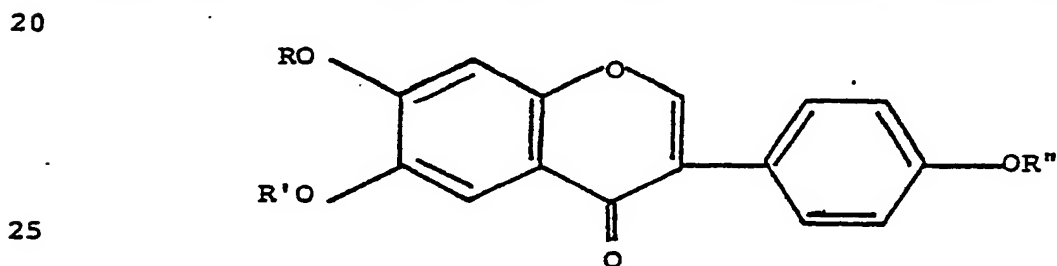
wherein R'' may be hydrogen or a methyl or ethyl group in dry ethyl-
ether containing zinc chloride, exposing said suspension to
dry hydrogen chloride for a sufficient time to form an oily
product, maintaining said suspension at an appropriate tem-
30 perature for a sufficient time to permit said oil product
formed upon exposure to dry hydrogen chloride to separate
from said suspension, removing and discarding the superna-
tant remaining after separation of said oil product from
said suspension, adding a major amount of water and a minor
35 amount of concentrated hydrochloric acid to said oil product
to form a mixture, boiling said mixture under reflux for a
sufficient time to produce a precipitate product, said pre-
cipitate product having the structure:

-31-

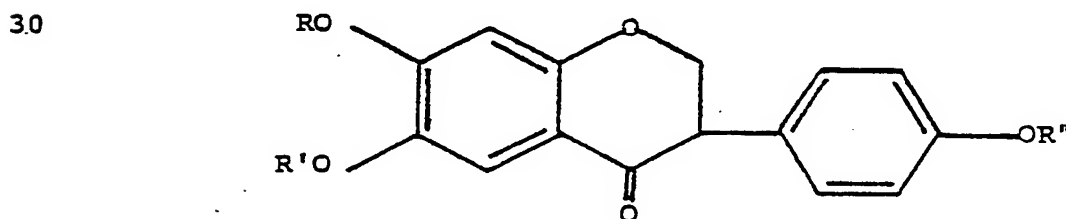


and recovering said precipitate;

dissolving said precipitate product in a suitable solvent,
 15 adding borontrifluoride-methyletherate, adjusting the temper-
 ature to about 50°C., adding methanesulphonyl chloride as a
 solution, heating the resulting solution at about 90-100°C.
 for a sufficient time to permit the reaction to proceed sub-
 stantially to completion, and recovering a compound having the structure:



30. A method of preparing a compound having the structure:



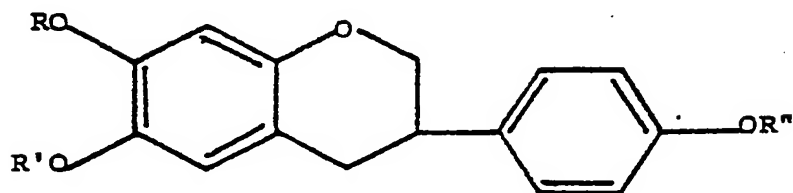
which comprises hydrogenating a compound in accordance with
 Claim 2.



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31. A method of preparing a compound having the structure:

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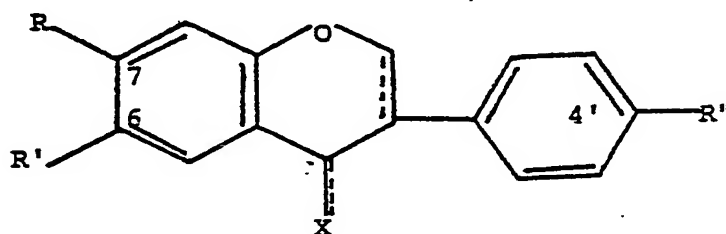


10 which comprises hydrogenating a compound in accordance with Claim 5.

32. A compound having the structure:

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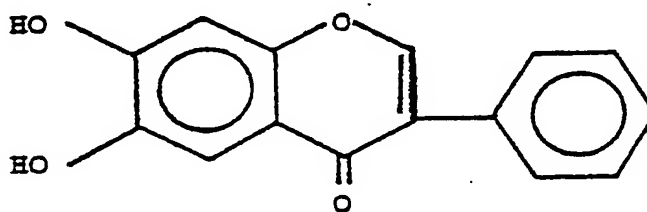


wherein the dashed lines may be carbon-carbon single bonds or carbon-carbon double bonds, and wherein X may be two hydrogen atoms or oxygen, and further wherein each of R, R' and R'' may be hydrogen, hydroxyl or a methyl or ethyl group.

33. A compound in accordance with Claim 32 having the structure:

30

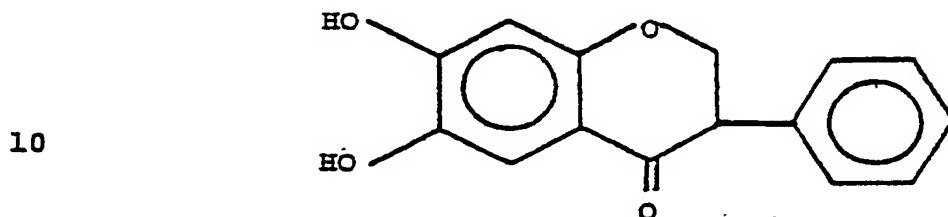
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-33-

34. A compound in accordance with Claim 32 having the structure:

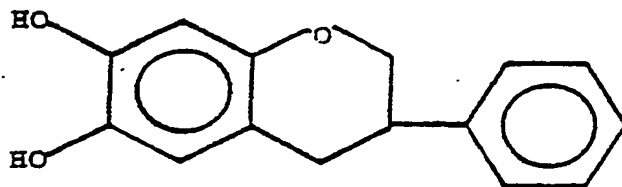
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35. A compound in accordance with Claim 32 having the structure:

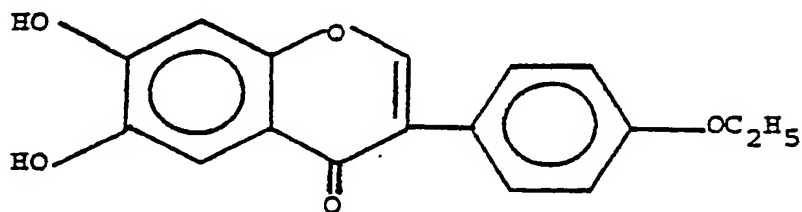
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36. A compound in accordance with Claim 32 having the structure:

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35

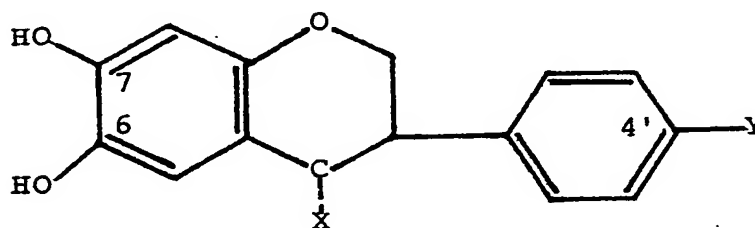
AMENDED CLAIMS

(received by the International Bureau on 16 May 1980 (16.05.80))

1. A compound having the structure:

5

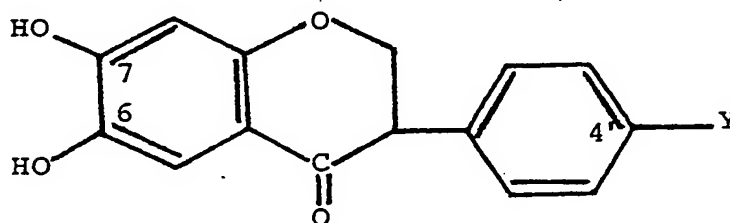
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wherein either the dashed line represents a carbon-carbon single bond and X represents two hydrogen atoms or the dashed line represents a carbon-carbon double bond and X represents oxygen, and wherein Y may be hydrogen or hydroxyl or a methoxy or ethoxy group.

2. A compound in accordance with Claim 1 having the structure:

25

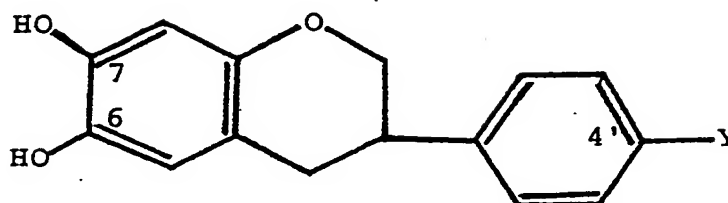


3. A compound in accordance with Claim 2 wherein Y is a methoxy group.

4. A compound in accordance with Claim 2 wherein Y is hydrogen.

35

5. A compound in accordance with Claim 1 having the structure:



6. A compound in accordance with Claim 5 wherein Y is a methoxy group.

15 7. A compound in accordance with Claim 5 wherein Y is hydrogen.

20 8. A antioxidant composition which comprises an effective amount of a compound in accordance with Claim 1 and a suitable carrier.

25 9. An antioxidant composition which comprises an effective amount of a compound in accordance with Claim 2 and a suitable carrier.

10. An antioxidant composition which comprises an effective amount of a compound in accordance with Claim 3 and a suitable carrier.

30 11. An antioxidant composition which comprises an effective amount of a compound in accordance with Claim 4 and a suitable carrier.

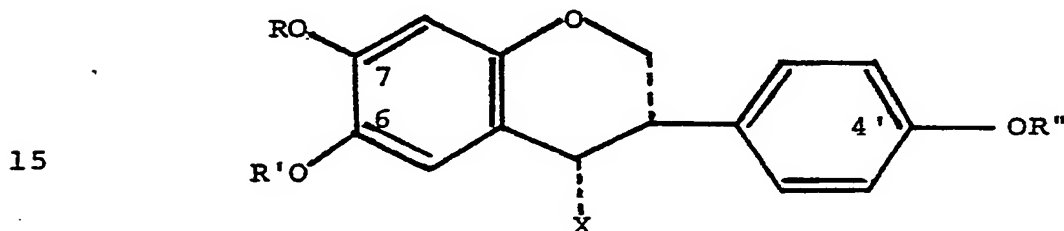
35 12. An antioxidant composition which comprises an effective amount of a compound in accordance with Claim 5 and a suitable carrier.



13. An antioxidant composition which comprises an effective amount of a compound in accordance with Claim 6 and a suitable carrier.

5 14. An antioxidant composition which comprises an effective amount of a compound in accordance with Claim 7 and a suitable carrier.

15 15. An antioxidant composition which comprises an effective amount of a compound having the structure:



20 wherein the dashed lines may be carbon-carbon single bonds or carbon-carbon double bonds and wherein X may be two hydrogen atoms or oxygen and further wherein each of R, R' and R'' may be a methyl or ethyl group or hydrogen.

25 16. An antioxidant composition in accordance with Claim 15 wherein in said compound said dashed lines are both carbon-carbon double bonds, X is oxygen, R and R'' are hydrogen and R' is methyl.

30 17. An antioxidant composition in accordance with Claim 15 wherein in said compound said dashed lines are both carbon-carbon double bonds, X is oxygen, R and R' are hydrogen and R'' is methyl.

35

18. A stabilized edible fat or oil composition which comprises an edible fat or oil and an antioxidant composition in accordance with Claim 8, said antioxidant composition being present in an amount effective to stabilize said
5 edible fat or oil.

19. A stabilized edible fat or oil composition which comprises an edible fat or oil and an antioxidant composition in accordance with any of Claims 9, 10, 11, 12, 13 or
10 14, said antioxidant composition being present in an amount effective to stabilize said edible fat or oil.

20. A stabilized edible fat or oil composition which comprises an edible fat or oil and an antioxidant composition in accordance with any of Claims 15, 16 or 17,
15 said antioxidant composition being present in an amount effective to stabilize said edible fat or oil.

21. A stabilized edible fat or oil composition in accordance
20 with Claim 18, wherein said edible fat or oil comprises animal fat or oil, vegetable oil, corn oil, cottonseed oil, lard, olive oil, soybean oil, bean oil, safflower oil, and polyunsaturated oils.

25 22. A stabilized edible fat or oil composition in accordance with Claim 18, wherein said antioxidant composition is present in an amount from about 0.01 percent to 1.0 percent by weight based upon the weight of said edible fat or oil.

30 23. A stabilized edible food product such as a fish or meat food product which comprises an edible food product and an antioxidant composition in accordance with Claim 8, said antioxidant composition being present in an amount effective to stabilize said edible food product.



24. A stabilized edible food product such as a fish or meat food product which comprises an edible food product and an antioxidant composition in accordance with any of Claims 9, 10, 11, 12, 13, 14, 15, 16 or 17, said antioxidant composition being present in an amount effective to stabilize said edible food product.

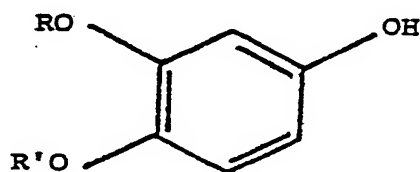
25. A method of preparing a compound in accordance with Claim 1 which comprises fermenting soybeans, contacting the resulting fermented soybean product with methanol to separate from said fermented soybean product an extract containing said compound and recovering said compound from said methanol extract.

26. A method in accordance with Claim 25 wherein said fermented soybean product is tempeh.

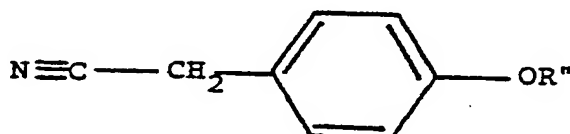
27. A method in accordance with Claim 25 wherein said extract containing said compound is obtained by contacting said fermented soybean product with an aqueous methanol solution at a temperature in the range about 0-10°C., evaporating the resulting methanol extract solution to dryness to produce a residue, redissolving a portion of said residue in dry methanol, separating and discarding the methanol insoluble portion of said residue, contacting said dry methanol solution containing said redissolved residue with hexane to remove contaminating material, discarding the resulting hexane extract, reducing the volume of the remaining methanol extract at a reduced temperature for 10-30 minutes so as to produce a precipitate, removing and discarding said precipitate, and recovering said compound from the remaining extract.

28. A method in accordance with Claim 25 wherein said recovery of said compound from said methanol extract comprises chromatographic separation, said chromatographic separation
5 being molecular sieve chromatography followed by adsorption chromatography.

29. A method of preparing a compound in accordance with Claim 2 which comprises forming a suspension of a compound
10 having the structure:



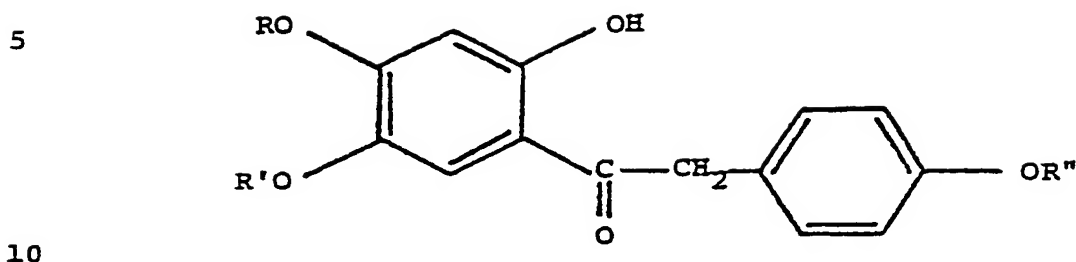
wherein each of R, R' and R'' may be either hydrogen or an ethyl or methyl group and a compound having the structure:



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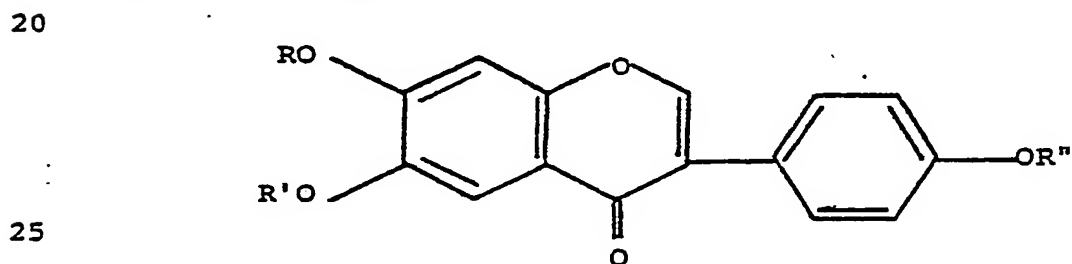
wherein R'' may be hydrogen or a methyl or ethyl group in dry ethyl-ether containing zinc chloride, exposing said suspension to dry hydrogen chloride for a sufficient time to form an oily product, maintaining said suspension at an appropriate temperature for a sufficient time to permit said oil product
30 formed upon exposure to dry hydrogen chloride to separate from said suspension, removing and discarding the supernatant remaining after separation of said oil product from said suspension, adding a major amount of water and a minor
35 amount of concentrated hydrochloric acid to said oil product to form a mixture, boiling said mixture under reflux for a sufficient time to produce a precipitate product, said precipitate product having the structure:



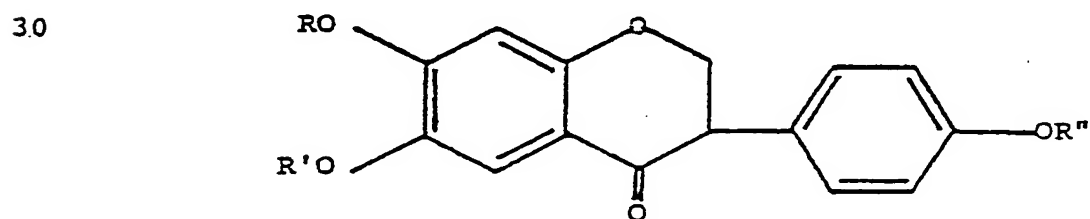


and recovering said precipitate;

dissolving said precipitate product in a suitable solvent,
 15 adding borontrifluoride-methyletherate, adjusting the temper-
 ature to about 50°C., adding methanesulphonyl chloride as a
 solution, heating the resulting solution at about 90-100°C.
 for a sufficient time to permit the reaction to proceed sub-
 stantially to completion, and recovering a compound having the structure:

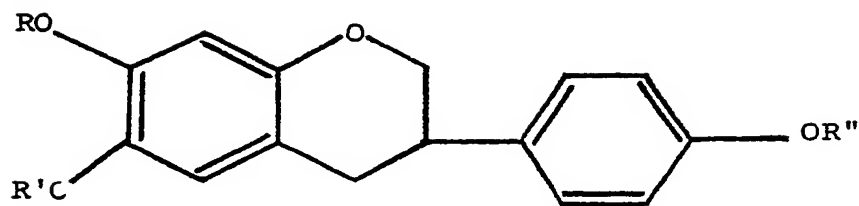


30. A method of preparing a compound having the structure:



which comprises hydrogenating a compound in accordance with
 Claim 2.

31. A method of preparing a compound having the structure:



which comprises hydrogenating a compound in accordance with Claim 15.

(32 to 36 cancelled)



INTERNATIONAL SEARCH REPORT

International Application No PCT/US79/00347

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL. A23B 5/14; A23D 5/04; C07D 311/04; 311/22; C09K 15/06; C12P 17/06
U.S. CL. 260/345.2, 345.5; 252/407; 426/545; 435/125

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System

Classification Symbols

US

260/345.2, 345.5; 424/545

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁵

Chemical Abstracts- Isoflavan, Isoflavanone and Isoflavone (1957-date).

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category *	Citation of Document, ¹⁶ with Indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X	US, A, 3,681,085 Published 1 August 1972	1-8, 17-24, 32-36
X	US, A, 3,762,933 Published 2 October 1973	1-8, 17-24, 32-36
X	N, Chemical Abstracts, 8th Collective Index, Volumes 66-75, Issued 1973 (Columbus, Ohio USA). See Pages 16,592S-16,596S. The Index inter alia, lists 4 isoflavans, 3 isoflavanones and 14 isoflavones within the scope of the claims	1-8, 32-36

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art

"E" earlier document but published on or after the International filing date

"L" document cited for special reason other than those referred to in the other categories

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but on or after the priority date claimed

"T" later document published on or after the International filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention

"X" document of particular relevance

IV. CERTIFICATION

Date of the Actual Completion of the International Search *

11 January 1980

Date of Mailing of this International Search Report *

15 FEB 1980

International Searching Authority ¹

ISA/US

Signature of Authorized Officer ²

NChan

Pucky Chan

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	N, Chemical Abstracts, 7th Collective Index, Volumes 56-65, issued 1969 (Columbus, Ohio, USA). See pages 12,259S-12,262S. The Index, inter alia, lists 3 isoflavans, 6 isoflavanones and 12 isoflavones within the scope of the claim s.	1-8 32-36
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V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹², specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

See the invitation to pay additional fees (PCT/ISA/206)
Mailed 16 January 1980.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.